Invasive *Carassius* spp. in the Tiber River basin (Umbria, Central Italy): population status and possible interactions with native fish species

by

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Abstract. – The purpose of the research was to analyse the distribution, abundance and growth of *Carassius* spp., a taxon which includes non-native species introduced into the water bodies of the Umbrian portion of the Tiber River basin during the late 1980s. Additionally, the study estimated the relative weight for *Barbus tyberinus*, *Squalius lucumonis* and *Sarmarutilus rubilio* to investigate the presence of competitive interactions between *Carassius* spp. and these native species that characterize the middle and lowland reaches of the Umbrian watercourses. Currently, *Carassius* spp. is widely distributed in both the stagnant and the slow-running waters of the entire basin. The presence of these species was recorded in 34.81% of the total sampling sites; the results showed that the presence of *Carassius* spp. was associated with the presence of other non-native species and with poor water quality. In total, 4520 specimens of *Carassius* spp. were collected and the age composition ranged from 0^+ to 9^+. The equation for the length-weight relationship (TL-W) was determined as $W = 0.010TL^{3.180}$. For the Etruscan chub *Squalius lucumonis*, an endemic species of central Italy, the mean value of the relative weight was significantly higher when *Carassius* spp. was absent. The results of the research suggest the need to establish effective management conservation strategies for this endemic species.

Résumé. – Les carassins invasifs, *Carassius* spp., dans le bassin du Tibre (Ombrie, Italie centrale) : état des populations et interactions possibles avec les espèces indigènes.

Le but de la recherche était d'analyser la distribution, l'abondance et la croissance du *Carassius* spp., un taxon qui comprend des espèces non indigènes introduites dans les plans d'eau de la partie ombrienne du bassin du Tibre à la fin des années 1980. En outre, l'étude a estimé le poids relatif de *Barbus tyberinus*, *Squalius lucumonis* et *Sarmarutilus rubilio* dans le but d'analyser la présence d'interactions concurrentielles entre *Carassius* spp. et les trois espèces indigènes qui caractérisent les tronçons moyens et terminaux des cours d'eau de l'Ombrie. Actuellement, *Carassius* spp. est largement distribué dans les eaux stagnantes et courantes de l'ensemble du bassin. La présence de ces espèces a été enregistrée dans 34,81% des sites d'échantillonnage ; les résultats ont montré que la présence du *Carassius* spp. a été associée à la présence d'autres espèces exotiques et à la mauvaise qualité de l'eau. Au total, 4520 spécimens de *Carassius* spp. ont été capturés et dix classes d'âge (de 0^+ à $9^{+)}$ ont été identifiées. La relation entre la longueur totale (TL) et le poids (W) est donnée par l'équation $W = 0{,}010TL^{3.180}$. Pour *Squalius lucumonis*, espèce endémique de l'Italie centrale, les valeurs moyennes du poids relatif étaient plus élevées dans les cas où *Carassius* spp. était absent. Les résultats de cette étude montrent la nécessité d'établir des stratégies efficaces pour la gestion de la conservation de cette espèce endémique.

There has been considerable confusion concerning the taxonomic status of the genus *Carassius*, due to the slight morphologic differences between the species (Rylkova *et al*., 2013). Currently in Europe is reported the presence of four species of the genus *Carassius*, namely *Carassius carassius* (Linnaeus, 1758), *Carassius auratus* (Linnaeus, 1758), *Carassius gibelio* (Bloch, 1782) and *Carassius langsdorfii* Temminck & Schlegel, 1846 (Kottelat and Freyhof, 2007).

The crucian carp *C. carassius* is native from Eastern Europe and most of Asia (Szczerbowsky, 2002); it was extensively imported to Italy, in many areas of England and France (Kottelat and Freyhof, 2007) in the mid 1800s (Szczerbowsky, 2002; Sarrocco *et al*., 2012). The goldfish *C. auratus* is reported to be native from East Asia and was introduced to Japan in the 16^{th} century (Kottelat and Freyhof, 2007). From Japan, it was imported to Europe (Szczerbowsky, 2002). The first introduction in Italy was dated back to the 17^{th} century (Bianco, 1995). The presence of both *C. carassius* and *C. auratus* was reported in Milan Province (Puzzi *et al*., 2007), in the Arno River basin (Tuscany Region) (Nocita, 2007) and in the southern part of the Tiber River basin (Lazio Region) (Tancioni and Cataudella, 2009; Sarrocco *et al*., 2012). The Prussian carp *C. gibelio* was generally considered native from central Europe and Siberia or imported in Europe from eastern Asia (Kottelat and Freyhof, 2007); Kalous *et al*. (2012) redescribed the species as native for Europe. Recent studies based on the genetic characterization showed the presence of the species in many European countries, including Italy (Rylkova *et al*., 2013). The Japanese species *C. langsdorfii* was detected in the last few years

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in the Elbe River basin in the Czech Republic (Kalous *et al.*, 2007), in Greece (Tsipas *et al.*, 2009; Rylkova *et al.*, 2013), in Bosnia-Herzegovina, Germany and Ukraine (Rylkova *et al.*, 2013); recent studies confirmed the presence of the species also in northwestern Italy (Kalous *et al.*, 2013; Rylkova *et al.*, 2013).

Based on diagnostic characters, as the number of gill rakers, the shape of the dorsal fin and the peritoneum colour (Balon, 2004; Kottelat and Freyhof, 2007), at first all the specimens considered in the present study were attributed to the species *C. auratus*. However, having not performed genetic analysis and considering: i) the high morphological similarity between *C. auratus*, *C. gibelio* and *C. langsdorfii*, and ii) the ease with which *C. auratus* can hybridize with *C. carassius* (Szczerbowsky, 2002), we cannot completely exclude the presence of other species in the sample. Therefore, in the present paper we used the scientific nomenclature of *Carassius* spp., which includes introduced species most probably of East Asian origin.

Relatively few ecological investigations on *Carassius* spp. populations have been carried out in Italy (Lorenzoni *et al.*, 2007; Pedicillo *et al.*, 2010). The presence of *Carassius* spp. in the water bodies of the Umbria region was reported for the first time in the late 1980s, and they easily placed themselves among the dominant species both in stagnant and slow-running waters. The presence of these species was detected in Trasimeno Lake in 1988, where it was most likely introduced stocked together with juvenile *Cyprinus carpio* Linnaeus, 1758 (Mearelli *et al.*, 1990; Ghetti *et al.*, 2007). *Carassius* spp., together with other numerous exotic species naturalized in Lake Trasimeno (Lorenzoni and Ghetti, 2012), have proven to be well adapted to the environmental characteristics of the lake; thus, their abundance have increased quickly, and they can be considered among the dominant species in the fish community (Mearelli *et al.*, 1990). Additionally, in the Corbara Reservoir, *Carassius* spp. is abundant and well acclimatized (Pedicillo *et al.*, 2010); during the monitoring carried out in 2000, *Carassius* spp. represented 30.79% of the total biomass of the fish caught (unpubl. data). In the watercourses, the presence of these species was detected for the first time in 1989, in the Chiascio River basin. Subsequently, *Carassius* spp. has rapidly spread, and in the past 27 years, the species has invaded many middle and lower reaches of the hydrographic system (Lorenzoni *et al.*, 2006, 2010a). The spontaneous expansion of *Carassius* spp. along the Tiber River and many of its tributaries has occurred using the connection of the hydrological networks (Lorenzoni *et al.*, 2007).

Among the fish species introduced into Italian freshwater, the species of the genus *Carassius* are considered among the most invasive and pose a serious threat to indigenous fish communities (Crivelli, 1995); species from Asia, in particular, seem to have a significant negative impact on freshwater ecosystems (Rylkova *et al.*, 2013). The characteristic that determines the invasiveness of *Carassius* spp. is mainly their great ability to tolerate extreme environmental conditions; they tolerate a high rate of pollution and can live in waters with a low percentage of oxygen (Szczerbowsky, 2002) and in extremely turbid waters (Crivelli, 1995; Balon, 2004). *C. auratus* in particular is tolerant to the high concentration of many heavy metals and organochlorine insecticides (Szczerbowsky, 2002). Of further importance is the wide food range and high fertility rate that characterize these species. One feature of *C. auratus* stocks in particular is the presence of triploid gynogenetic females and diploid females that reproduce sexually (Buth *et al.*, 1991). *Carassius auratus* populations, with a highly unbalanced sex ratio in favour of females, were found in Italian inland waters, including the middle reaches of the Po River (females: 98.00-100.00%) (Vitali and Braghieri, 1981) and in Lake Trasimeno (females: 97.40%) (Lorenzoni *et al.*, 2010b). A further determinant factor for the success of these species is represented by their high growth rate that allows it to quickly reach a size that is large enough to escape predators (Lorenzoni *et al.*, 2007).

In many places where they have been introduced, *Carassius* spp. proved to have a negative impact on existing native species; they are considered a threat to the native fish communities and are subject to containment programmes (Ghetti *et al.*, 2007). With regard to the habitat alteration, previous research showed that the presence of abundant populations of *C. auratus* in lakes causes a notable increase of the water turbidity (Crivelli, 1995; Richardson *et al.*, 1995). This increase in turbidity could be due to the movement of the sediment that occurs when the goldfish feed or to the increase of predation on zooplankton, which results in an increase of phytoplankton and, thus, the eutrophication of the lake waters (Crivelli, 1995). In Lake Trasimeno, the increase of *C. auratus* abundance (73.23% of the total catch during a monitoring carried out in 2004) has been related to the decrease of the abundance of the endemic species *Esox cisalpinus* (Bianco & Delmastro, 2011), a predator that needs transparent waters to sight its prey (Lorenzoni *et al.*, 2010c); other species, as *Cyprinus carpio* and *Tinca tinca* (Linnaeus, 1758), contribute minimally to the increase of water turbidity, as they currently are characterized by low abundance values (Lorenzoni and Ghetti, 2012). The benthic feeding of *C. auratus* is able to keep the nutrients suspended, making them available to algae and contributing to their proliferation (Morgan *et al.*, 2004). Moreover, the omnivorous diet of *C. auratus* includes eggs, fry and adults of native fishes (Morgan *et al.*, 2004).

In this context, it was important to investigate the possible interactions of invasive exotic species, such as the *Carassius* spp., with native fish species in the study area. The purposes of this research were to analyse the distribution, abundance and growth of *Carassius* spp. in the Umbrian

section of the Tiber River basin. Another aim was to evaluate the possible impact of the species on the native fish communities, by estimating the relative weight for three endemic species of the Umbrian watercourses in the presence and absence of *Carassius* spp.

MATERIALS AND METHODS

The investigated area includes the watercourses in the Umbrian portion of the Tiber River basin for an area of 8412 km^2, equal to 66.28% of the total extension (Fig. 1) (Lorenzoni *et al.*, 2006). This area was divided into the following five sub-basins: the Chiascio, the Nera, the Nestore, the Paglia and the residual section of the Tiber River, which included the main course and other minor tributaries. A total of 158 sampling sites located on 77 watercourses were included in the study area. The analyses were carried out using the data of the Regional Fish Map of the 1st and 2nd levels and the 1st update, for fish collected during the following periods: 1990-1996, 2000-2006 and 2007-2014.

A census of the fish fauna was carried out in each sampling site using the removal method (Moran, 1951; Zippin, 1956). The fish were captured at the low-flow period using a continued or pulsed direct current electric shocker, with the power varying between 1.500 and 4.500 W. All the captured fishes were identified and counted. For all the specimens, the total length (TL) was measured to the nearest 0.1 cm, and

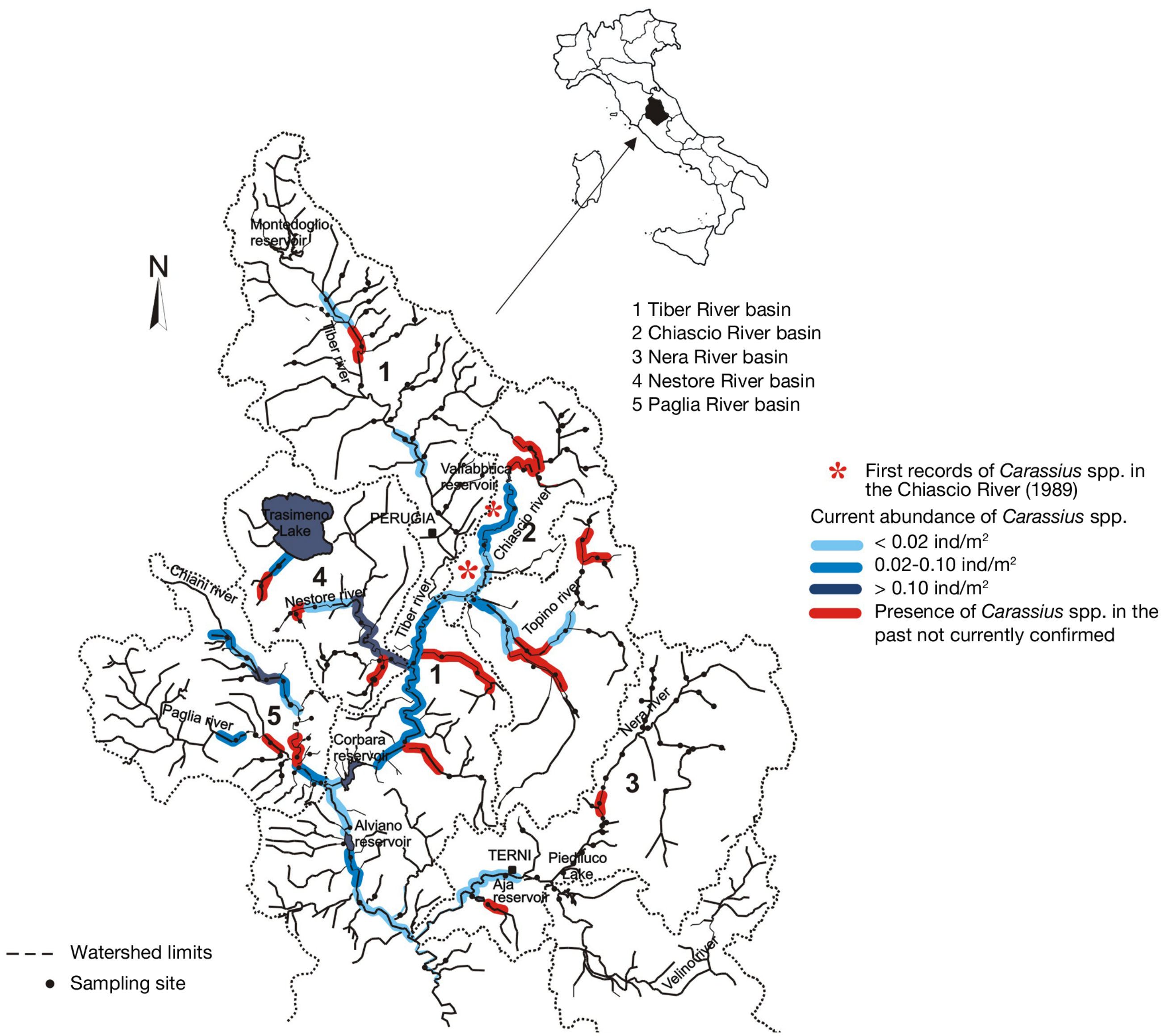

Figure 1. – Study area, location of the sampling sites, current and past distribution and current abundance of *Carassius* spp.

Table I. – *U* Mann-Whitney test analysis: comparison of the environmental parameters for the sampling sites in which *Carassius* spp. were present, and those in which they were absent. $p < 0.05$ is in bold.

	Carassius spp. absence		*Carassius* spp. presence					
Environmental parameters	N values	Mean (± SD)	N values	Mean (± SD)	Min.	Max.	*Z* values	*p*
Distance from the source (km)	573	21.39 ± 29.38	105	60.39 ± 61.58	0.13	216.00	8.175	**0.000**
Watershed area (km^2)	573	176.1 ± 388.89	105	703.90 ± 1256.46	7.74	6349.70	7.901	**0.000**
Altitude (m a.s.l.)	578	312.35 ± 140.48	110	192.12 ± 83.94	42.00	480.00	9.095	**0.000**
Average slope (%)	575	2.18 ± 1.81	111	1.84 ± 1.45	0.12	7.20	1.395	0.163
pH (units)	578	8.10 ± 0.28	111	8.02 ± 0.33	6.48	8.92	2.689	**0.007**
Conductivity (mS cm^{-1} at 25°C)	579	570.50 ± 187.12	111	749.49 ± 264.55	353.00	2220.00	8.342	**0.000**
BOD_5 (mg l^{-1})	357	1.58 ± 1.53	76	4.23 ± 4.51	0.60	26.00	7.235	**0.000**
COD (mg l^{-1})	333	7.97 ± 7.82	70	13.34 ± 11.57	0.33	75.00	5.320	**0.000**
NNO_3 (mg l^{-1})	375	1.72 ± 3.06	82	2.06 ± 2.35	0.05	11.00	2.306	**0.021**
NNO_2 (mg l^{-1})	375	0.03 ± 0.08	82	0.08 ± 0.16	0.00	0.91	5.366	**0.000**
NNH_3 (mg l^{-1})	579	0.28 ± 1.41	111	0.45 ± 1.33	0.00	12.30	4.004	**0.000**
SO_4 (mg l^{-1})	578	51.64 ± 54.74	111	89.73 ± 75.53	6.10	523.00	7.277	**0.000**
PPO_4 (mg l^{-1})	505	0.06 ± 0.10	100	0.14 ± 0.27	0.00	1.82	3.787	**0.000**
P tot (mg l^{-1})	343	0.07 ± 0.12	72	0.20 ± 0.38	0.00	2.20	3.911	**0.000**
Cl (mg l^{-1})	578	19.64 ± 21.42	111	33.50 ± 27.57	7.00	162.00	7.803	**0.000**
Water temperature (°C)	567	13.73 ± 4.16	107	16.39 ± 5.06	3.00	26.00	5.683	**0.000**
Dissolved oxygen (mg l^{-1})	570	9.43 ± 1.67	109	9.23 ± 2.43	1.97	17.00	1.142	0.254
Extended Biotic Index (EBI)	565	7.57 ± 1.58	112	6.54 ± 1.21	3.00	9.00	7.452	**0.000**
EBI Quality Class	565	2.46 ± 0.83	112	3.01 ± 0.68	2.00	5.00	6.432	**0.000**
Width (m)	314	6.23 ± 0.5.75	62	12.30 ± 8.98	1.05	43.00	6.001	**0.000**
Depth (m)	301	0.35 ± 0.21	59	0.41 ± 0.34	0.08	1.70	0.390	0.697
Average current speed (m s^{-1})	538	0.28 ± 0.27	90	0.27 ± 0.23	0.03	1.39	0.384	0.701
Flow rate (m^3 s^{-1})	547	1.53 ± 10.11	97	1.27 ± 1.64	0.01	7.07	5.069	**0.000**
Wetted river section (m^2)	532	1.96 ± 5.62	91	3.53 ± 3.74	0.05	19.76	6.439	**0.000**
Average shaded surface (units)	377	2.34 ± 1.40	82	1.30 ± 1.22	0.00	4.00	5.811	**0.000**
Cover (units)	378	2.49 ± 1.13	80	2.09 ± 1.12	0.00	4.00	2.918	**0.004**
Canopy cover (units)	373	0.89 ± 1.00	80	1.21 ± 1.27	0.00	4.00	1.770	0.077
Substrate size (units)	383	4.99 ± 2.01	83	3.80 ± 2.40	1.00	7.00	4.185	**0.000**

the weight was measured to the nearest 0.1 g (Anderson and Neumann, 1996). A sample of the scales was collected from each specimen for age determination. At the end of the field activities, all the fish caught were released into their natural environment. All scales were stored in ethanol (33%) and later observed under a stereomicroscope using the image-analysis system IAS 2000. Fish age was determined by the scalimetric method using two operators (Bagenal, 1978) and was further validated through the analysis of the length-frequency distribution (Britton *et al*., 2004). For all sites, the stream reach length was established as 10 times the wetted channel, with a minimum and maximum length of 50 to 400 m. Twenty-eight environmental parameters were used to characterize the river sectors (Tab. I). The Extended Biotic Index (EBI) (Ghetti, 1986) is a biotic index used to evaluate overall water quality based on the sensitivity to pollution of some key groups of the macrobenthic fauna: clean streams are given an index of 15, and this value decreases as pollution increases. The hydrologic variables were measured at transects within each sampling reach. The watershed area, the distance from the source, the average slope and the altitude were determined from Istituto Geografico Militare (IGM) topographic maps. Electronic instruments were used for the field measurements of the specific conductivity, the pH, the water temperature and the dissolved oxygen. The other chemical parameters of the water were determined according to APHA, AWWA and WPCF (1989) and APAT, CNR and IRSA (2003) specifications. The substrate size was evaluated according to the diameter of the main component among those present in the sampling site. For each size category, an index value ranging from 1 (< 1 mm) to 7 (> 256 mm) was given. The cover, the canopy cover and the shaded surface were estimated by visual observations using a five-degree scale, as follows: 0 = absent, 1 = isolated areas, 2 = frequent interruptions, 3 = few interruptions, and

4 = continuous areas. The environmental parameters were usually assessed on the same day as the fish samplings.

The total length-weight relationship (TL-W) was estimated by the least-squares method (Ricker, 1975) based on the following logarithmic equation:

$$\log_{10} W\ (g) = a + b \log_{10} TL\ (cm)$$

The theoretical growth was estimated by the von Bertalanffy growth-curve model (von Bertalanffy, 1938):

$$TL_t = L_\infty(1 - e^{-k(t - t_0)})$$

where TL_t is the total length of the fish at time t, L_∞ is the theoretical maximum length (cm), k is the rate of approach to L_∞, and t_0 is the theoretical age at which $TL_t = 0$. Additionally, the index of growth performance (Φ') was calculated by the equation of Pauly and Munro (1984):

$$\Phi' = \log10\ k + 2 \log10\ L_\infty$$

where k and L_∞ are the growth parameters of the von Bertalanffy model.

The length-weight relationship and the theoretical growth calculations were also carried out using the data recorded in 2006 and in 2014 for the *Carassius* spp. populations of the Corbara Reservoir and Lake Trasimeno, respectively.

The level of the quality change in fish community was assessed through the Zoogeographic Integrity Coefficient (ZIC) (Bianco, 1990; Elvira, 1995), which is calculated as the ratio between the number of indigenous species and the total number of species identified. This index ranged from one (no non-native species present) to zero (all non-native species present).

To extract the environmental variables driving the observed fish assemblages, the Canonical Correspondence Analysis (CCA) (ter Braak, 1986) was performed. The CCA analysis was processed with the CANOCO statistical package for Windows 4.5. The results of the CCA generate a diagram that displays the approximate values of the weighted averages of the fish assemblage parameters (points) with respect to the supplied environmental variables. In the diagram, the environmental variables are represented by arrows that point in the direction of the maximum factor variation (ter Braak, 1986). The position of the points in relationship to the arrows indicates the relationship between each point and the variable represented by the arrow. The points that are farthest along towards the head of the arrow represent the largest values for that variable. To assess the statistical significance of the ordination axis, we ran Monte Carlo tests for 1000 permutations. An axis was considered statistically significant if the eigenvalue from the randomly permuted set exceeded the original in 50 or fewer cases, $\alpha = 0.05$. To minimize the potential biases affecting the CCA due to the differences between periods and the differences in the sampling sites and to make the data collected in the three census periods comparable, the fish and environmental data sets were restricted to 125 sites. Each site was sampled once during each of the three census periods. The environmental matrix used included 15 variables and 375 observations (sites × sampling events). The fish assemblage matrix that was used included 18 variables (7 native fish species and 11 non-native ones) and 375 observations. Non-native fish species have been selected based on the results of previous research that showed the close link of these species with the presence of *Carassius* spp. in the study area (Carosi *et al*., 2015); the seven native species are the key species that characterize the middle and lower stream reaches of the Tiber River basin (Lorenzoni *et al*., 2010a). The abundance of a fish species was coded using a scale that varies from 0 to 3 based on the population density (0 ind/m^2 = absent; < 0.05 ind/m^2 = rare; from 0.05 to 0.1 ind/m^2 = sub-dominant; > 0.1 ind/m^2 = dominant). To fit a statistical model of change in the densities of *Carassius* spp. and other species densities along axis 1, a Generalized Linear Model (GLM) was used to model the species response by means of normal distribution.

To assess the interaction of *Carassius* spp. with the native species, the body condition of *Barbus tyberinus* Bonaparte, 1839, *Sarmarutilus rubilio* (Bonaparte, 1837) and *Squalius lucumonis* (Bianco, 1983) was estimated. The two reasons for the choice of these endemic species were abundance values that were high enough to enable the analysis to be conducted and their status as species of conservational interest. Thirty-nine sampling sites were selected to estimate the body condition based on their close link with the presence of *Carassius* spp., as shown by the species-sampling sites plot of the CCA analysis.

The body condition was estimated by the relative weight W_r:

$$W_r = 100(W / W_s)$$

where W = weight (g); W_s = standard weight.

The relative weight (W_r) is a body condition index based on a comparison between the real weight of an individual and the ideal weight of a specimen of the same species of the same length in good physiological condition (W_s) (Brown and Murphy, 1991). In the present study W_s was computed separately for each species of interest using the following equations reported in literature, calculated with the empirical percentile (EmP) method using a wide sample of specimens collected throughout its distribution range (Angeli *et al*., 2010; Giannetto *et al*., 2012a):

B. tyberinus:
$\log_{10}(W_s) = -4.917 + (2.987 \log_{10}LT) - 0.003(\log_{10}LT)^2$

S. lucumonis:
$\log_{10}(W_s) = -7.750 + 5.750 \log_{10}LT - 0.660 (\log_{10}LT)^2$

S. rubilio:
$\log_{10}(W_s) = -4.086 + 1.864 \log_{10}LT) + 0.351(\log_{10}LT)^2$

W_r values lower than the optimal range (95-105) indicate poor body condition (Murphy *et al*., 1990; Blackwell *et al*., 2000). W_r allows to quickly evaluate the physiological sta-

tus of fish (Brown and Murphy, 1991) and to evidence the occurrence of ecological changes (Blackwell *et al.*, 2000).

As already tested in a previous study (Carosi *et al.*, 2016), to play down the influence of the presence of other non-native species, the analysis was carried out by i) choosing the sampling locations with a close association between *Carassius* spp. and the indigenous species, and ii) selecting only the sampling locations with similar environmental features to exclude the potential effect of the longitudinal gradient.

RESULTS

The total sample was composed of 4520 specimens of *Carassius* spp., including 775 from 21 rivers of the Tiber River basin, 2985 from Lake Trasimeno and 760 from the Corbara Reservoir. The size of the collected fish ranged in total length from 2.00 to 49.50 cm (mean ± SE = 10.12 ± 0.15), and its weight ranged from 0.40 to 2185.96 g (mean ± SE = 326.60 ± 4.89). Ten age classes from 0^+ to 9^+ were identified.

Distribution and abundance

The results of the fish census showed that *Carassius* spp. was present in 55 sampling sites (34.81% of the total) and was widely distributed in the Tiber River and its main tributaries, which include Chiascio, Topino, Nestore, and Paglia Rivers. In the Nera River basin, the presence of *Carassius* spp. was limited to the south-central part of the watershed (Fig. 1). In some stream reaches, mainly located in the upper part of the Tiber and Chiascio basins, the presence of the species was occasional in the past and has not been confirmed in recently. Higher density values were reported for the Nestore River basin (average density value: 0.043 ind/m^2) and for the Tiber River basin (average density value: 0.038 ind/m^2). Much lower density values characterized the Nera River basin (average density value: 0.007 ind/m^2) and the Chiascio River basin (average density value: 0.011 ind/m^2). In the Nestore River and in the Paglia River basins, a considerable increase of the density values over time was observed (Fig. 2); the results showed an opposite trend for the three other basins. The differences were not statistically significant for the two-way factorial analysis of variance ($F = 1.86$; $p = 0.06$).

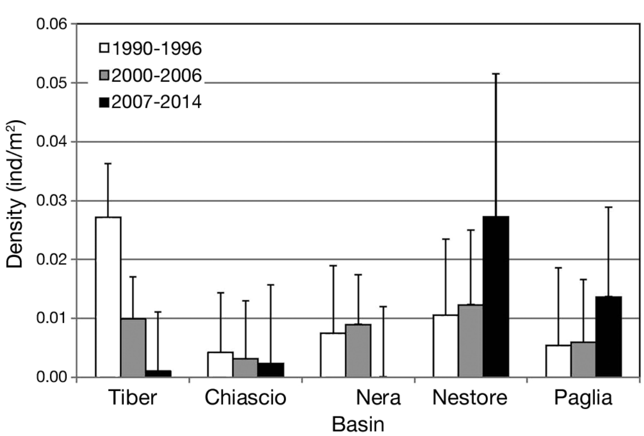

Figure 2. – Comparison of the mean population density values for *Carassius* spp. between basins in the three census periods. The vertical bars represent the 95% confidence level.

Environmental characterization

The sites where *Carassius* spp. was present were characterized by high contents of dissolved salts in the water, as shown by the high value of conductivity (mean value: 749.49 mS cm^{-1}) and by high concentrations of sulphates (average value: 89.73 mg l^{-1}) and chlorides (average value: 33.50 mg l^{-1}) (Tab. I). The low quality EBI values (average: 2.90) confirmed that the presence of *Carassius* spp. was associated with poor water quality. The watershed areas were comprised within a large range from 7.74 to 6349.70 km^2. The water temperature fluctuated over a wide range between 3°C to 26°C; the same wide range of values can be observed for the dissolved oxygen content, which fluctuated from 1.97 to 17.00 mg l^{-1}. Water pH ranged from 6.48 to 8.92 units. Flow-rate values ranged from a minimum of 0.01 to a maximum of 7.74 m^3 s^{-1}. The river locations in which the species was present were also characterized by low values of the shaded surface and the canopy cover. The differences between the mean values calculated in the sampling sites in which *Carassius* spp. were present and those in which they were absent, were statistically significant for the *U* Mann-Whitney test analysis for all the environmental parameters except the average slope, the dissolved oxygen, the depth, the average current speed and the canopy cover (Tab. I).

Fish community

A total of 41 fish species were found (Tab. II). The results of the CCA allowed the relationships between the

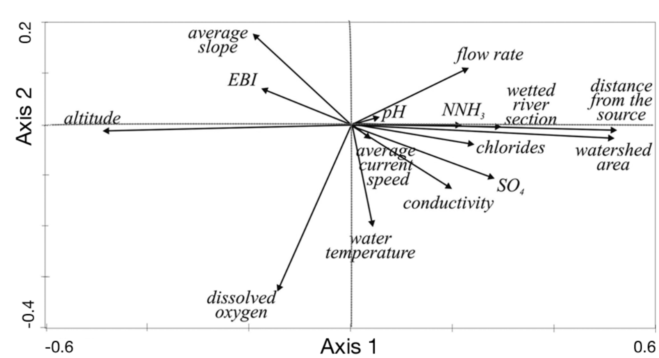

Figure 3. – Canonical Correspondence Analysis plot of the environmental variables. The arrows represent the vectors of the environmental variables. The length of the arrow is proportional to the importance of each variable: a long arrow indicates large spatial changes closely correlated with the ordination axes.

Table II. – Updated list of species found in the study area according to recent revisions of Bianco (2014) and Bianco and Ketmaier (2014), and their origin. For native species is also reported the threatened status according to IUCN RED LIST criteria for Italy (Rondinini *et al*., 2013), and year of introduction for alien species in the Tiber basin and reference, when available.

Family	Species	Common name	Origin	IUCN category for native and years of introduction for non-native species
Anguillidae	*Anguilla anguilla* (Linnaeus, 1758)	Eel	Native	CR
Cyprinidae	*Alburnus arborella* (Bonaparte, 1841)	Padanian bleak	Non-native	1960 (Lorenzoni *et al*., 2006)
	Barbus barbus (Linnaeus, 1758)	Barbel	Non-native	1998 (Mearelli *et al*., 2000)
	Barbus plebejus Bonaparte, 1839	Padanian barbel	Native	VU
	Barbus tyberinus Bonaparte, 1839	Tiber barbel	Native	VU
	Blicca bjoerkna (Linnaeus, 1758)	White bream	Non-native	2008 (unpubl. data)
	Carassius spp.		Non-native	1989 (Lorenzoni *et al*., 2006)
	Chondrostoma soetta Bonaparte, 1840	Padanian nase	Non-native	Before 1989 (Bianco, 1989)
	Ctenopharyngodon idellus (Valenciennes, 1844)	Grass carp	Non-native	1986 (Mearelli *et al*., 1990)
	Cyprinus carpio Linnaeus, 1758	Carp	Non-native	1710 (Ghetti *et al*., 2007)
	Gobio gobio (Linnaeus, 1758)	Gudgeon	Non-native	1999 (unpubl. data)
	Leucos aula Bonaparte, 1841	Padanian roach	Non-native	1960 (unpubl. data
	Luciobarbus graellsii (Steindachner, 1866)	Iberian barbel	Non-native	2010 (Buonerba *et al*., 2013)
	Protochondrostoma genei (Bonaparte, 1839)	Italian nase	Non-native	1960 (Sommani, 1967)
	Pseudorasbora parva (Temminck & Schlegel, 1846)	Top mouth gudgeon	Non-native	1994 (Lorenzoni *et al*., 1997)
	Rhodeus sericeus (Pallas, 1776)	Bitterling	Non-native	2003 (unpubl. data)
	Rutilus rutilus (Linnaeus, 1758)	Roach	Non-native	2004 (La Porta *et al*., 2010)
	Sarmarutilus rubilio (Bonaparte, 1837)	Apennine roach	Native	NT
	Scardinius hesperidicus Bonaparte, 1845	Padanian rudd	Non-native	1988 (Bianco, 1994)
	Squalius lucumonis (Bianco, 1983)	Etruscan chub	Native	CR
	Squalius squalus (Bonaparte, 1837)	Italian chub	Native	LC
	Telestes muticellus (Bonaparte, 1837)	Italian riffle dace	Native	LC
	Tinca tinca (Linnaeus, 1758)	Tench	Non-native?	Unknown, probably Middle Age (Bianco and Ketmaier, 2014)
Centrarchidae	*Lepomis gibbosus* (Linnaeus, 1758)	Pumpkinseed	Non-native	1926 (Mearelli *et al*., 1990)
	Micropterus salmoides (Lacepède, 1802)	Largemouth bass	Non-native	1989 (Bianco, 1989)
Cobitidae	*Cobitis bilineata* Canestrini, 1865	Padanian Spined loach	Non-native	1989 (Bianco, 1994)
Cottidae	*Cottus gobio* Linnaeus, 1758	Bullhead	Native	LC
Esocidae	*Esox lucius* Linnaeus, 1758	Northern pike	Non-native	Unknown, probably last century
	Esox cisalpinus Bianco & Delmastro, 2011	Southern pike	Native	DD
Gasterosteidae	*Gasterosteus aculeatus* Linnaeus, 1758	Three-spined stickleback	Native	LC
Gobiidae	*Padogobius bonelli* (Bonaparte, 1846)	Padanian goby	Non-native	1993 (Lorenzoni *et al*., 1997)
	Padogobius nigricans (Canestrini, 1867)	Arno goby	Native	VU
Ictaluridae	*Ameiurus melas* (Rafinesque, 1820)	Black bullhead	Non-native	1988 (Ghetti *et al*., 2007)
Percidae	*Perca fluviatilis* Linnaeus, 1758	Perch	Non-native	1920 (Mearelli *et al*., 1990)
	Stizostedion lucioperca (Linnaeus, 1758)	Pikeperch	Non-native	1964 (Sommani, 1967)
Poeciliidae	*Gambusia holbrooki* Girard, 1859	Eastern mosquitofish	Non-native	1922 (Sommani, 1967)
Salmonidae	*Oncorhynchus mykiss* (Walbaum, 1792)	Rainbow trout	Non-native	–
	Salmo trutta (complex) Linnaeus, 1758	Brown trout	Native/Non-native	–
	Salvelinus fontinalis (Mitchill, 1814)	Brook trout	Non-native	2006 (Lorenzoni *et al*., 2010a)
	Thymallus thymallus (Linnaeus, 1758)	Grayling	Non-native	Around 1967 (Sommani, 1967)
Siluridae	*Silurus glanis* Linnaeus, 1758	Wels catfish	Non-native	Around 1993 (Bianco, 1994)

fish species densities and the environmental variables to be highlighted through the reorganization of the total variability. The first two axes explained 73.70% of the overall variability ($p = 0.001$; total inertia = 2.17). The first axis of the CCA explained 62.20% of the total variability ($F = 34.33$; $p = 0.001$). All the environmental variables, except the average current speed, were significantly correlated with this axis, which effectively described the changes that occurred

along the longitudinal gradient of the rivers (Tab. III). Specifically, a progressive decline in the water quality (decrease of the EBI and dissolved oxygen values, increase of the chlorides and sulphates) seems to be associated with an increase in the river size (increase of the wetted river section, distance from the source and watershed area) (Fig. 3). The second axis of the CCA was less informative (11.50% of the overall variability) and reflected the inverse correlation between the conductivity and the water temperature with the average slope of the watercourses.

The plot of the fish matrix (Fig. 4) showed that *Carassius* spp. was positioned in the lower stream reaches, where its presence was closely linked with that of other non-native species, as follows: *Cyprinus carpio*, *Lepomis gibbosus* (Linnaeus, 1758), *Pseudorasbora parva* (Temminck and Shlegel, 1846) and *Protochondrostoma genei* (Bonaparte, 1839). The middle stream reaches of the hydrographic system were mainly characterized by rheophilic cyprinids and other fish species typical of the barbel zone: *B. tyberinus*, *Squalius squalus* (Bonaparte, 1837), *Padogobius nigricans* (Canestrini, 1867), *S. lucumonis* and *S. rubilio*. In the lowland zone of the rivers, at the left end of the distribution, the most limnophilous non-native species were located, which included *Rhodeus sericeus* (Pallas, 1776), *Rutilus rutilus* (Linnaeus, 1758) and *Scardinius hesperidicus* (Bonaparte, 1842); except for *S. hesperidicus*, all these species have been introduced into the study area in relatively recent times, specifically, between 1998 and 2004. The relationship between the spe-

Table III. – Canonical Correspondence Analysis (CCA): canonical and correlation coefficients of environmental variables with axis. $p < 0.05$ is in bold.

	Canonical coefficients		Correlations with axis			
Environmental parameters	AX1	AX2	AX1	p	AX2	p
Distance from the source (km)	0.195	–0.009	0.5657	**0.001**	–0.1075	0.113
Watershed area (km^2)	0.192	–0.004	0.5724	**0.001**	–0.1700	**0.012**
Altitude (m a.s.l.)	–0.303	–0.082	–0.5038	**0.001**	–0.0648	0.340
Average slope (%)	–0.077	0.159	–0.4247	**0.001**	0.1268	0.061
pH (units)	–0.071	0.183	–0.1701	0.120	0.0957	0.158
Conductivity (mS·cm^{-1})	0.062	–0.077	0.3112	**0.001**	–0.0058	0.933
NNH_3 (mg·L^{-1})	0.085	–0.044	0.1867	**0.006**	–0.0680	0.316
SO_4 (mg·L^{-1})	0.119	–0.146	0.3762	**0.001**	–0.0008	0.991
Cl (mg·L^{-1})	0.050	0.068	0.2475	**0.001**	0.0779	0.251
Water temperature (°C)	0.037	–0.052	0.2360	**0.001**	–0.0333	0.624
Dissolved oxygen (mg·L^{-1})	–0.167	–0.305	–0.2782	**0.001**	–0.0056	0.934
E.B.I. (units)	–0.019	0.032	–0.2413	**0.001**	0.0880	0.194
Average current speed (m·s^{-1})	–0.040	–0.129	–0.0023	0.973	–0.0834	0.219
Flow rate (m^3·s^{-1})	–0.059	0.361	0.2725	**0.001**	–0.1571	**0.020**
Wetted river section (m)	0.151	–0.258	0.4569	**0.001**	–0.3095	**0.001**

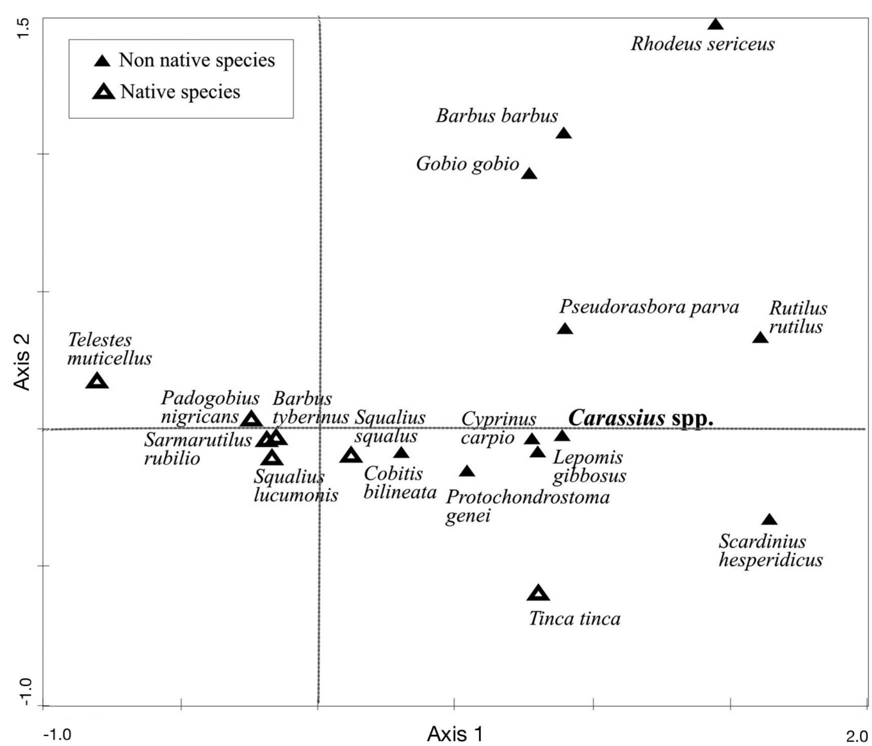

Figure 4. – Canonical Correspondence Analysis: plot of the densities of native and non-native species. The eigenvalues of axes 1 and 2 were 0.249 and 0.046, respectively. The first two axes explained 73.70% of the total variance.

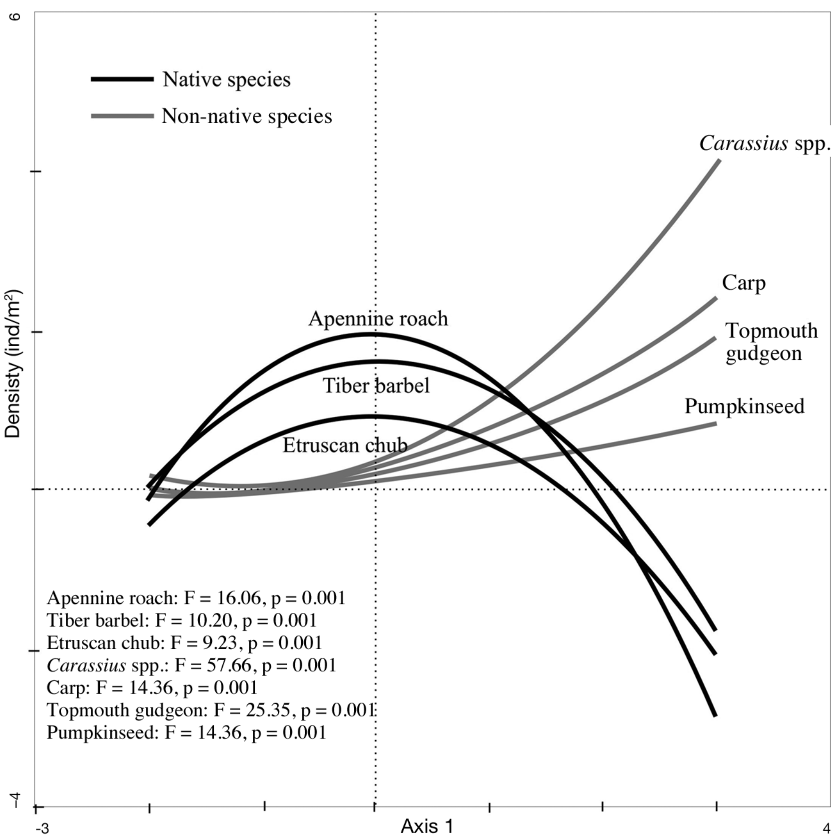

Figure 5. – Trend of three native and four non-native species densities along axis 1 of the Canonical Correspondence Analysis, evaluated through Generalized Linear Model analysis.

Table IV. – *U* Mann-Whitney test analysis for total number of fish species, number of native and non-native fish species and ZIC: comparison of sampling sites in which *Carassius* spp. were present and absent. $p < 0.05$ is in bold.

	Carassius spp. absence		*Carassius* spp. presence					
	N values	Mean (± SD)	N values	Mean (± SD)	Min.	Max.	Z	*p*
Total number of fish species	584	4.418 ± 2.95	112	9.35 ± 3.16	2.00	17.00	12.518	**0.000**
Number of native fish species	584	3.351 ± 2.00	112	4.54 ± 1.80	0.00	8.00	5.547	**0.000**
Number of non-native fish species	584	1.067 ± 1.71	112	4.79 ± 2.65	0.00	13.00	13.125	**0.000**
ZIC	584	0.876 ± 0.26	112	0.54 ± 0.21	0.14	1.00	12.355	**0.000**

cies densities and axis 1, evaluated through the GLM analysis, showed that the increasing abundance of the goldfish and the most closely related non-native species associated along the longitudinal gradient, corresponds to a progressive decrease in the densities of the native species (Fig. 5). For all the species, the relationship was statistically significant. In the lower stream reaches, the occurrence of many exotic species and the poor water quality resulted in a decrease in the component of the native fish communities, as revealed by the low mean value of the ZIC in the sampling sites in which *Carassius* spp. was present (0.54) (Tab. IV). The differences between the mean values calculated in the sampling sites with and without *Carassius* spp. were highly statistically significant for the *U* Mann-Whitney test analysis for the total number of fish species, the number of indigenous fish species, the number of exotic fish species and the ZIC.

Growth

The TL-W relationship for the total sample was:

$$W = 0.010TL^{3.180 \pm 0.004}\ (R^2 = 0.99).$$

The b (slope) value of the TL-W regressions was significantly greater than 3 (t = 45; p = 0.001), indicating positive allometric growth (Ricker, 1975).

The parameters of the theoretical growth were calculated separately for the Corbara Reservoir and Trasimeno Lake (Tab. V). From the comparison of the two populations, the growth for the *Carassius* spp. of the Corbara Reservoir can be considered faster and shows better performance.

Table V. – Parameters of theoretical growth for *Carassius* spp. of the Corbara Reservoir and Trasimeno Lake.

	TL_t	k	t_0	R^2	Φ'
Trasimeno Lake	63.52	0.135	–0.811	0.99	2.74
Corbara Reservoir	55.95	0.208	–0.316	0.99	2.81

Table VI. – *U* Mann-Whitney test analysis: comparison of the Wr mean values calculated for the presence and absence of *Carassius* spp. with three native species. $p < 0.05$ is in bold.

Wr	Mean syntopy	Mean allotopy	*Z* value	*p*	N Values syntopy	N Values allotopy	SD syntopy	SD allotopy
Sarmarutilus rubilio	99.036	99.032	0.496	0.620	1041	2528	19.932	21.046
Squalius lucumonis	92.660	102.050	2.405	**0.016**	90	349	16.950	17.140
Barbus tyberinus	92.817	93.494	0.946	0.344	786	1976	17.150	24.151

Body condition

The W_r mean values calculated for both the presence (syntopy) and absence (allotopy) of *Carassius* spp. and for the species *S. rubilio*, *S. lucumonis,* and *B. tyberinus* were compared (Tab. VI). For *S. lucumonis* and *B. tyberinus,* the average values of the relative weight were higher in cases where *Carassius* spp. was absent; the differences between the mean values were statistically significant for the *U* Mann-Whitney test analysis only for *S. lucumonis* (Z = 2.405; p = 0.016).

DISCUSSION

In the study area, the presence of *Carassius* spp. was reported for the first time in the end of the 80s, when the species were found in Lake Trasimeno and in two sampling sites located in the middle course of the Chiascio River and one of its tributaries, Saonda Creek (Mearelli *et al*., 1996).

The rapid expansion of these species had a detrimental effect on the native fish community and represented a serious threat in a Mediterranean area such as the Umbria region, with its high incidence of endemic species with limited distributions. The principal threat to indigenous fish species is most likely competition for food and other resources (Lorenzoni *et al*., 2010a). It has been reported in the literature that the goldfish introduced into European water bodies affected native fish, such as carp, the crucian carp *C. carassius* and the tench *Tinca tinca* (Halačka *et al*., 2003); in addition, pike *Esox lucius* Linnaeus, 1758 may decrease in abundance as a result of the increased water turbidity (Cowx, 1997). Also in Lake Trasimeno, the decline of the endemic southern pike *E. cisalpinus* has been related to the increase of *C. auratus* (Lorenzoni *et al*., 2010c). In this lake, *Carassius* spp., in addition to interacting negatively with the most important species of interest from an economic viewpoint, are species of low commercial interest, and their current high abundance has a negative impact on professional fishing because the massive presence of *Carassius* spp. often makes it difficult to catch fish and to collect fish from nets.

However, on the basis of the present results, the negative effects of the presence of *Carassius* spp. appeared to be less evident in running waters than in stagnant waters. Although the species is widely distributed throughout the entire hydrographic network, in any of the five basins examined, high density values of *Carassius* spp. were detected. The disappearance over time of the species from the upstream reaches can be attributed to their limnophylic characteristics, which make it unsuitable to rheophilic environments, as the Nera River and the upper sections of the Tiber and the Chiascio rivers. By comparison with these areas in terms of abundances, a contrasting trend over time was observed for the Nestore and the Paglia River basins: the torrential characteristics of the streams, with marked flow rate oscillations and the occurrence of drought periods in summer, make these environments more suitable for the limnophilous and more tolerant species. The most abundant populations of *Carassius* spp. have been detected in the Nestore River basin, an area that is characterized by the high presence of human activities that strongly affect water quality. In the study area, the presence of *Carassius* spp. was detected in stream reaches with low values of dissolved oxygen (1.97 mg l^{-1}) and high values of conductivity, in accordance with reports for English populations of *C. auratus* (Copp *et al*., 2010). The wide ranges of water temperature values (3-26°C) confirmed the high tolerance of *Carassius* spp. to extreme environmental conditions, according to the thermal limits reported in literature, as 0-38.5°C for *C. carassius* (Szczerbowsky, 2002). The low mean value of the EBI class quality (average: 2.90) showed that the presence of *Carassius* spp. in the study area was associated with water pollution.

The CCA analysis showed that *Carassius* spp. were positioned in the lowland zone of the rivers, as was also reported for an Australian river (Morgan and Beatty, 2007). In that area, the presence of many exotic species and the low environmental quality resulted in a deterioration of the quality of

the fish communities (Lorenzoni *et al*., 2006), as shown by the low mean value of the ZIC in the sampling sites in which *Carassius* spp. was present (0.54). The presence of *Carassius* spp. in the Tiber River basin was associated with that of other non-native species, such as *C. carpio, L. gibbosus* and *P. parva*. As clearly shown by the GLM analysis, the densities of these species increased along the longitudinal gradient, while the native species densities decreased.

The maximum estimated age (9^+) is higher than that reported in previous research carried out in the study site; for the populations of Trasimeno Lake and Corbara Reservoir, the oldest specimens have been attributed to the age class 7^+ (Lorenzoni *et al*., 2010b, 2010c; Pedicillo *et al*., 2010). Other studies have indicated a maximum age of 41 years (Froese and Pauly, 2010). The specimens of maximum size (total length = 49.50 cm; weight = 2185.96 g) exceeded the maximum total length previously reported for *C. auratus* (48.00 cm) (Froese and Pauly, 2010) and the maximum size of 35 cm and 1 kg reported for goldfish, Crucian carp and Prussian carp by Balon (2004).

The higher growth resulted for *Carassius* spp. of the Corbara Reservoir can be attributed to the different ecological conditions compared to Lake Trasimeno, in terms of greater food availability and lower population density.

Carassius spp. and the other non-native species showed markedly limnophilic characteristics and preferably colonize the lowland areas of the rivers. Although the native species preferentially colonize the more upstream reaches than the most polluted areas inhabited by the goldfish, frequently a co-presence of these species was observed. In these cases negative impacts on native species may be observed. As previous research had shown (Giannetto *et al*., 2011, 2012b; Carosi *et al*., 2016), the estimate of the body condition for the native species was effective in assessing the impact of *Carassius* spp., since it allows to show the presence of competitive interaction phenomena. The results obtained with this method, which still needs further field experimentation and testing, suggested that the presence of *Carassius* spp. could have a negative impact on *S. lucumonis*. This endemic species of central Italy is of particular conservational interest; the distribution areal of *S. lucumonis* is restricted to three river basins in central Italy (Giannetto *et al*., 2013). In the red lists of the IUCN (Rondinini *et al*., 2013), *S. lucumonis* is listed as “species critically endangered”. The presence of the goldfish did not seem to affect the condition of the Tiber barbel and the Apennine roach.

Further analysis, based on the genetic characterization, are required to clearly identify the occurrence of *Carassius* species in the Tiber River basin. The information collected in this study is the basis for undertaking proper management actions for the conservation of native biodiversity and to adopt specific eradication programmes to control unwanted species, such as *Carassius* spp.

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Annexe 1. – Georeferenced *Carassius* spp. record data from the Regional Fish Map of the Umbria Region.

Basin	Water body	Site code	Geographic coordinate
Chiascio	Chiascio River	01CHIA04	33T 307515 4791405
	Chiascio River	01CHIA06	33 T 305516 4783835
	Chiascio River	01CHIA07	33T 299855 4776753
	Chiascio River	01CHIA08	33 T 300513 4772114
	Chiascio River	01CHIA09	33 T 291092 4766531
	Clitunno River	01CLIT02	33 T 312755 4752866
	Rasina River	01RASI01	33 T 309991 4789034
	Saonda River	01SAON01	33 T 308535 4795100
	Timia River	01TIMI01	33 T 304102 4758939
	Topino River	01TOPI01	33 T 321755 4774806
	Topino River	01TOPI04	33 T 315409 4761731
	Topino River	01TOPI05	33 T 307428 4757511
	Topino River	01TOPI06	33 T 303309 4762969
	Topino River	01TOPI07	33 T 297156 4766797
Nera	Aia River	02AIAA01	32 T 299666 4710073
	Aia River	02AIAA02	32 T 297752 4710922
	Nera River	02NERA07	33 T 321548 4728426
	Nera River	02NERA11	33 T 304627 4715134
Nestore	Anguillara River	03ANGU01	33 T 260814 4771614
	Calvana River	03CALV01	33 T 278862 4750889
	Calvana River	03CALV02	33 T 280547 4753455
	Ierna River	03IERN02	33 T 264014 4763472
	Moiano River	03MOIA01	33 T 257399 4765385
	Nestore River	03NEST03	33 T 267325 4764684

Basin	Water body	Site code	Geographic coordinate
	Nestore River	03NEST04	33 T 277198 4761744
	Nestore River	03NEST05	33 T 282375 4755421
Paglia	Nestore River	04ASTR01	33 T 252740 4757910
	Chiani River	04CHIA01	33 T 255100 4755293
	Chiani River	04CHIA02	33 T 258917 4750617
	Chiani River	04CHIA03	33T 262133 4750911
	Chiani River	04CHIA04	33 T 263821 4746355
	Chiani River	04CHIA06	33 T 265201 4735412
	Paglia River	04PAGL01	33 T 742255 4739893
	Paglia River	04PAGL03	33 T 260276 4738699
	Paglia River	04PAGL05	33 T 270378 4730869
Tiber	Grande d'Amelia River	06GRAA01	33 T 283462 4706532
	Naia River	06NAIA03	33 T 288570 4738390
	Puglia River	06PUGL01	33 T 298480 4753401
	Puglia River	06PUGL02	33 T 292316 4755881
	Tiber River	06TEVE01	33 T 270334 4823263
	Tiber River	06TEVE02	33 T 277316 4813370
	Tiber River	06TEVE04	33 T 285626 4796023
	Tiber River	06TEVE06	33 T 290577 4765301
	Tiber River	06TEVE07	33 T 286440 4754385
	Tiber River	06TEVE08	33 T 285369 4739552
	Tiber River	06TEVE09	33 T 274355 4723364
	Tiber River	06TEVE10	33 T 276850 4709477
	Tiber River	06TEVE11	33 T 289737 4697311